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Indirect haemagglutination test using gonococcal pilus antigen: how useful to diagnose gonorrhoea?

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SUMMARY In 1979 an indirect haemagglutination test (gonococcal antibody test) using gonococcal pilus antigen replaced the gonococcal complement fixation test as our routine procedure to show gonococcal antibodies.

In the diagnosis of current gonorrhoea the sensitivity of the gonococcal antibody test was far superior to that of the gonococcal complement fixation test (about 55% versus 9% for first episode gonorrhoea).

To evaluate the usefulness of the test result the following population groups were studied: 1376 patients undergoing medical examination for gonorrhoea (386 had gonorrhoea), 1384 healthy people aged 15–65, 54 patients with meningococcal disease, 30 children with respiratory tract infection, and 254 patients with evidence of various diseases other than neisserial infections that might be associated with symptoms of arthritis. These investigations showed that (1) non-specific positive gonococcal antibody test results occur rarely, (2) at least half the people who have had gonorrhoea remain seropositive (with titres of 1/40 to 1/160), and (3) a positive test result is more significant the younger the patient and the higher the titre. For younger people a positive test result should always be followed up by bacteriological examination; in all age groups titres of 1/320 or more should indicate medical examination for current gonorrhoea.

At the Neisseria department of the Statens Seruminstitut, a gonococcal complement fixation test was performed routinely for 50 years.[1] When the gonococcal complement fixation test was introduced its sensitivity for diagnosing current gonorrhoea was 44–56% in patients with uncomplicated infection and 69–100% in those with complicated infection.[2] Investigations performed in 1973 showed a sensitivity of only 2–5% for patients with uncomplicated infection and 16–30% for patients with complicated infection.[3 4] Though the gonococcal complement fixation test could no longer be considered useful for clinical purposes, 90 000 analyses a year were still requested. Based on the promising results obtained by Buchanan *et al* using gonococcal pilus antigen,[5] the task of developing a new serological test was undertaken.[6 7] Since 1979 an indirect haemagglutination test (gonococcal antibody test) using gonococcal pilus as antigen has been applied routinely. The department now receives about 20 000 specimens a year from Denmark and Greenland.

In this paper we describe the results obtained by examination of sera from patients visiting venereal disease clinics, other groups of patients with diseases that may give symptoms of arthritis, patients with meningococcal disease, and healthy people examined for pharyngeal carriage of *Neisseria meningitidis*. We also investigated the antibody status in healthy people aged over 15.

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Patients and methods

STUDY POPULATION

We studied sera from the following groups of people: (1) 1376 patients undergoing medical examination for gonorrhoea comprising 649 patients visiting a dermatovenereological outpatient day clinic in Copenhagen, of whom 184 (already described[7]) had gonorrhoea confirmed by culture, and 727 patients visiting another venereological outpatient clinic in Copenhagen, of whom 202 had gonorrhoea; (2) 1384

healthy people comprising 160 aged 15–28 who had been examined for pharyngeal carriage of meningococci, 598 blood donors aged 18–39, and 626 blood donors aged 40–65; and (3) 338 patients suffering from diseases other than gonorrhoea, especially diseases associated with symptoms of arthritis. This group comprised 30 children with respiratory disease aged up to 12, 54 patients with meningococcal disease who gave positive results in the meningococcal complement fixation test (15 were children aged up to 12), 102 patients with Reiter's syndrome,[8] 67 patients with rheumatoid arthritis, 45 patients giving positive test results for *Yersinia enterocolitica* (type 3) antibodies, 20 patients giving positive Widal reactions, and 20 patients infected with bacteriologically identified *Campylobacter* spp.

BACTERIOLOGICAL DIAGNOSIS

Secretions from the urethra, cervix (of women), rectum, and fauces were collected on charcoal impregnated swabs and submitted to the laboratory in modified Stuart's medium. In these studies the transportation time never exceeded six hours. Each specimen was inoculated on to both selective and non-selective medium, and bacteria from gonococcus like, oxidase positive colonies were identified as being *N gonorrhoeae* by a direct immunofluorescence test.[9] Throat isolates suspected of being gonococci or meningococci were further identified by Gram stain and carbohydrate utilisation tests.

PILUS ANTIGEN PREPARATION

The pili were purified according to the method of Hermodsen *et al*[10] with a few modifications. We have been purifying pili according to various methods and from different strains, but we so far prefer *N gonorrhoeae* F 62 for producing pilus antigen. The strain and the method of purifying pili were chosen because of their excellent yield of pilus antigen and because the antigenic properties of F 62 pilus antigen do not differ from those of pilus antigens of most other gonococcal strains.

INDIRECT HAEMAGGLUTINATION TEST (GONOCOCCAL ANTIBODY TEST)

The test was performed as described previously.[6 7] The highest serum concentration was 1/20. Serum was diluted two fold from a dilution of 1/20. Titres of 1/40 were considered to show positive test results.

GONOCOCCAL COMPLEMENT FIXATION TEST AND MENINGOCOCCAL COMPLEMENT FIXATION TEST

The antigens were prepared and the tests performed as described by Reyn.[11] The highest serum concentration tested was 1/12. The titre was read as the lowest serum concentration giving 60% haemolysis.

STATISTICAL METHODS

Sensitivity, specificity, and the predictive value of positive and negative test results were defined as described by Vecchio.[12]

Results

Promising preliminary results in evaluating the gonococcal antibody test for diagnosing gonorrhoea[6 7] were achieved when only one highly skilled technician performed the test. To adapt the method to semi-automatic performance, and to train technicians to perform the test routinely, we obtained sera from 727 patients attending a venereological outpatient clinic. The results were similar to those found earlier[7]; few (8% (2/25) to 11% (9/85)) patients with current gonorrhoea were seropositive in the gonococcal complement fixation test, whereas most (51% (24/47) to 88% (22/25)) were seropositive in the gonococcal antibody (indirect haemagglutination) test (table 1). A 58% specificity of the gonococcal antibody test means that 42% (221) of 526 patients without current gonorrhoea were found to be seropositive; 53% (266/504) of men and 38% (85/223) of women studied gave histories of gonorrhoea.

Tables 2 and 3 show concentrations of antibodies in the 1376 patients examined for gonorrhoea. Table 2 shows that 47% (47) of 101 men and 61% (71) of 116 women with first episode gonorrhoea were seropositive, and 10% of the men and 16% of the women in this group had titres of 1/320 or more. Of patients with current gonorrhoea who had previously had gonorrhoea, 92% (36/39) of women and 79% (102/130) of men were seropositive; and 44% of the women and 25% of the men in this group had titres of 1/320 or more.

Table 3 shows the concentrations of antibodies in sera from the 990 patients who did not have gonorrhoea at the time of examination. Of the 557 who had no history of gonorrhoea, 21% (114) were seropositive and 3% had titres of 1/320 or more. Of the 433 who had a history of gonorrhoea, 47% (205) were seropositive.

Fig 1 shows the yearly numbers of reported cases of gonorrhoea in Denmark from 1900; 10–25% of people born before 1960 had gonorrhoea at least once. We calculated the incidence of seropositive people as a percentage of those born in any year on the assumption that 30%, 40%, 50%, or 60% of patients with gonorrhoea remain seropositive (fig 2). The calculations were based on the following data and assumptions: (1) the yearly number of cases of gonorrhoea reported, (2) number of people born in a particular year who were alive when aged 15, (3) that all cases notified a year were new cases (which means that the number of unreported cases was considered

Table 1 *Serological investigation of 223 women and 504 men attending venereological outpatient clinic in Copenhagen (1978)*

Patients with current gonorrhoea			*Sensitivity (%) of:*		*Predictive value (%) of positive result in:*	
Sex	*No*	*History of gonorrhoea*	*GAT*	*GCFT*	*GAT*	*GCFT*
Women	25	Yes	88	8	36	100
Women	44	No	57	9	48	57
Men	85	Yes	81	11	41	75
Men	47	No	51	9	30	67
Total	201		70	9	39	70
Patients without current gonorrhoea			*Specificity (%) of:*		*Predictive value (%) of negative result in:*	
Sex	*No*	*History of gonorrhoea*	*GAT*	*GCFT*	*GAT*	*GCFT*
Women	60	Yes	35	100	88	72
Women	94	No	71	97	78	69
Men	181	Yes	44	98	83	70
Men	191	No	71	99	85	81
Total	526		58	98	83	74

GAT = gonococcal antibody test (using gonococcal pilus antigen). GCFT = gonococcal complement fixation test (using whole cell antigen).

Table 2 *Concentrations of antibodies to gonococcal pilus antigen (GAT titres) in sera from 386 patients with current gonorrhoea attending venereological outpatient clinics in Copenhagen*

	No (%) without history of gonorrhoea				*No (%) with history of gonorrhoea*			
GAT titre	*Women (n = 116)*		*Men (n = 101)*		*Women (n = 39)*		*Men (n = 130)*	
⩽ 1/20	45	(39)	54	(54)	3	(8)	28	(22)
1/40	21		13		2		28	
1/80	23	(45)	9	(37)	5	(49)	29	(54)
1/160	8		15		12		13	
1/320	10		7		6		11	
1/640	3		2		3		12	
1/1280	4	(16)	0	(10)	6	(44)	6	(25)
1/2560	1		0		2		1	
1/5120	1		1		0		2	
⩾ 1/40	71	(61)	47	(47)	36	(92)	102	(79)

GAT = gonococcal antibody test.

Table 3 *Concentrations of antibodies to gonococcal pilus antigen (GAT titres) in sera from 990 patients without current gonorrhoea attending venereological outpatient clinics in Copenhagen*

	No (%) without history of gonorrhoea				*No (%) with history of gonorrhoea*			
GAT titre	*Women (n = 200)*		*Men (n = 357)*		*Women (n = 122)*		*Men (n = 311)*	
⩽ 1/20	159	(80)	284	(80)	64	(53)	164	(53)
1/40	15		36		13		38	
1/80	17	(19)	16	(17)	18	(38)	51	(37)
1/160	6		8		15		26	
1/320	3		8		6		15	
1/640	0		1		6		7	
1/1280	0	(2)	4	(4)	0	(10)	10	(10)
1/2560	0		0		0		0	
1/5120	0		0		0		0	
⩾ 1/40	41	(21)	73	(20)	58	(48)	147	(47)

GAT = gonococcal antibody test.

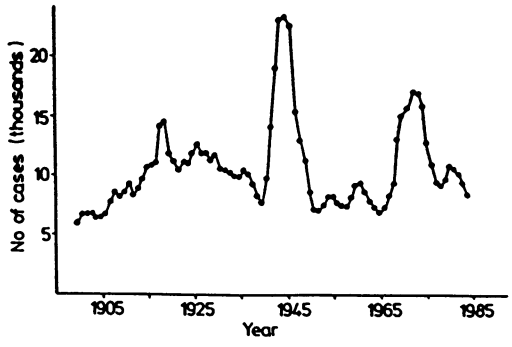

Fig 1 *Yearly number of cases of gonorrhoea reported to the Danish National Health Service.*

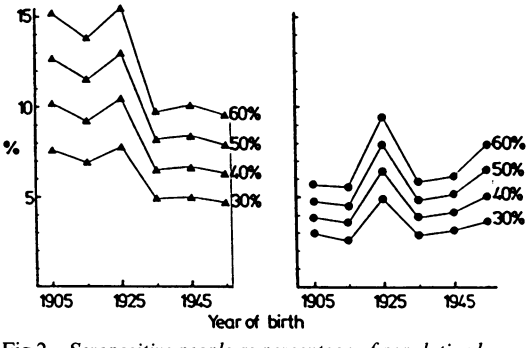

Fig 2 *Seropositive people as percentage of population born in a particular year if 30%, 40%, 50%, or 60% of notified patients with gonorrhoea are assumed to remain seropositive for life (▲ men, ● women).*

to be equivalent to the number of "repeaters"), and (4) that the age distribution of those who contracted gonorrhoea was the same in each year, most women contracting the disease when aged 19, and most men when aged 22.

Table 4 shows the concentrations of gonococcal antibodies obtained by testing sera from 1384 people from the general population. Seropositive people made up 7% (17/253) of women and 7% (35/505) of men aged under 40 and 7% (11/166) of women and 12% (53/460) of men aged 40 or more. These percentages agreed with the calculated values illustrated in fig 2 if 40–50% of men and more than 60% of women who contract gonorrhoea are assumed to remain seropositive. It is noteworthy that the titres were almost always low (1/40 to 1/160), and only 1% (12/1384) had titres of 1/320 or more.

To investigate the specificity of a positive gonococcal antibody test result we tested sera from 54 patients with meningococcal disease and from 160 healthy people examined for pharyngeal carriage of *N meningitidis*. Sera from 10 adults and two children with meningococcal disease and positive meningococcal complement fixation test results were also positive in the gonococcal antibody test. Sera from four of the adults and both children gave negative results in the meningococcal complement fixation and gonococcal antibody tests on admission to hospital, but were positive in both tests later (at low titres in the gonococcal antibody test). Six sera out of 29 from meningococcal carriers were positive in the meningococcal complement fixation test, two were weakly positive in the gonococcal antibody test, and only one of these gave positive results in both tests. Of the 131 sera from non-carriers, 11 were positive in the meningococcal complement fixation test and five in the gonococcal antibody test (at low titres). None gave positive results in both tests simultaneously. No information of current or previous sexually transmissible disease was available.

Sera from 30 children aged 12 or younger who had respiratory tract infection all gave negative results in the gonococcal antibody test.

Because requests for the gonococcal antibody test were often for patients of 40 or older with arthralgia, we also tested sera from patients with evidence of various diseases that might be associated with symptoms

Table 4 *Concentrations of antibodies to gonococcal pilus antigen (GAT titres) in sera from 1384 healthy people in the general population*

	No (%) aged under 40				*No (%) aged 40 and over*			
GAT titre	*Women (n = 253)*		*Men (n = 505)*		*Women (n = 166)*		*Men (n = 460)*	
≤ 1/20	236	(93)	472	(94)	155	(93)	405	(88)
1/40	7		19		4		24	
1/80	7	(6)	11	(7)	3	(6)	14	(10)
1/160	1		4		3		7	
1/320	1		1		0		3	
1/640	1		0		1		4	
1/1280	0	(1)	0	(0)	0	(1)	1	(2)
1/2560	0		0		0		0	
1/5120	0		0		0		0	
≥ 1/40	17	(7)	35	(7)	11	(7)	53	(12)

GAT = gonococcal antibody test.

Table 5 *Concentrations of antibodies to gonococcal pilus antigen (GAT titres) in sera from patients with diseases associated with reactive arthritis*

Sera from patients with:	*No*	*Age in years*	*No of seropositive patients with GAT titre of:*		
			1/40–1/160	*⩾1/320*	*Total ⩾1/40*
Reiter's syndrome*	102	17 to 65	8	4	12
Rheumatoid arthritis†	67	17 to 80 (median 62)	10	1	11
Yersinia enterocolitica (type 3) antibodies†	45	Unknown	0	0	0
Positive Widal reaction†	20	Unknown	0	0	0
Campylobacter spp infection†	20	2 to 60	0	0	0

*Eight out of 12 seropositive patients had either current or previous gonorrhoea.
†No information available about current or previous sexually transmitted disease.

of arthritis, such as Reiter's syndrome, rheumatoid arthritis, and *Campylobacter* spp infection, and from patients who were seropositive for *Yersinia enterocolitica* (type 3) antibodies or in the Widal test (table 5). There was no indication that cross reacting antibodies are induced in people with these conditions.

Discussion

In the developed countries excellent facilities for bacteriological diagnosis of gonorrhoea exist in many places. Procedures to show gonococcal antibodies are still of interest, however, because culture is unreliable when patients have received antibiotics before being examined and in patients with pelvic inflammatory disease or other manifestations of disseminated gonococcal infection.

In the diagnosis of acute infection the gonococcal antibody test was found to be much more sensitive than the gonococcal complement fixation test and at least as sensitive as serological tests investigated by others,[5 13–15] though 46% of patients attending STD clinics with first episode gonorrhoea were seronegative. If a serological test is to be used to detect cases of current gonorrhoea in an unselected population, Galen and Gambino[16] claim that the minimum requirements of the test should be: (1) sensitivity greater than 80%, (2) specificity greater than 99%, and (3) the ability to differentiate current from past infection. So far, no test to show gonococcal antibodies fulfills all these conditions.[17–19]

The specificity of serological tests to diagnose gonorrhoea depends firstly on the availability of a gonococcal antigen that is unrelated to antigens of other micro-organisms, especially those associated with other sexually transmissible diseases and with diseases that have symptoms similar to those of disseminated gonococcal infection. We found no evidence that non-specific positive gonococcal antibody test results occur in these conditions. Seroconversion (from negative to titres of 1/40 to 1/160) has, however, been noted in a few patients with meningococcal disease. About half the patients without current gonorrhoea who had a history of gonorrhoea were seropositive. This finding prompted us to investigate the antibody status of the general population. We found that 8% (116/1384) were seropositive. In Denmark 10–25% of people born between 1900 and 1960 have had gonorrhoea. Thus it could be concluded that 40–50% of men and more than 60% of women who contract gonorrhoea remain seropositive, presumably for life. The test of choice for diagnosing current gonorrhoea is therefore still culture. Furthermore, culture is always recommended to confirm the diagnosis when gonorrhoea is suspected on the basis of a positive gonococcal antibody test result. The use of serology as the only diagnostic tool should be limited to patients whose case history and clinical picture are consistent with gonococcal infection and for whom the reliability of diagnosis by culture is low.

Judging from numerous telephone conversations with doctors from all over the country during the past six years, it must frankly be admitted that the gonococcal antibody test is used in an unsatisfactory way. Knowledge of the use of serology and how to interpret serological test results seems to be difficult to achieve. Beebe investigated the use of a gonococcal antibody screening test by doctors in New Jersey.[20] Despite a thorough bulletin, stressing target groups and the proper use and interpretation of the test, being enclosed with each kit, the doctors used the test on patient groups other than those recommended, and the test results were interpreted as one would interpret the results of culture. They did not realise that the test results are likely to be negative in the early stages of infection and that antibody to *N gonorrhoeae* is usually long lived, even after successful treatment.

Our conclusion, after six years of experience with the gonococcal antibody test is that the test is quick and easy to perform, fairly sensitive, and superior in specificity, but its usefulness is hampered by the

absence of antibodies in the early stages of disease and by the persistence of antibodies in about half the patients contracting gonorrhoea. It is useful for diagnosis in selected groups of patients, especially those with pelvic inflammatory disease and other disseminated gonococcal infections. The test might be used for screening younger patients, especially women, who often have asymptomatic infections. The younger the patient and the higher the titre, the more significant is a positive test result. In young patients a positive gonococcal antibody test result should always be followed by culture. In any age group, titres of 1/320 or more should be followed up by bacteriological examination. Finally, we feel strongly that it is mandatory to increase education about how to use serology in medical decision making.

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